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14. ABSTRACT As an approach to identify potential Shh-responding stem cells in the mouse prostate, we used Genetic Inducible Fate Mapping (GIFM) to follow the fate of Shh-responding cells both during prostate development and during androgen-mediated regeneration of the gland in the adult, two processes that are driven by stem or progenitor cell expansion. As <i>Gli1</i> expression is a sensitive readout of Shh signaling, we used a <i>Gli1<sup>CreER</sup></i> allele and <i>Rosa26</i> reporter to fate map Shh-responding cells. We show that Shh-responding cells do not expand over time in the normal homeostatic prostate, but these same cells do expand massively after androgen-mediated regeneration, indicating that Shh-responding cells are normally quiescent, but retain the ability to expand in the adult prostate. The expansion of cells is confined to stromal fibroblasts and smooth muscle cells; no glandular epithelial cells are marked. These results indicate that <i>Gli1</i> either specifically marks stromal stem cells that expand during regeneration to give rise to the two stromal cell types, or that fibroblasts and smooth muscle cells in general have a high capacity for proliferation even in the adult prostate. To determine whether the marked Shh-responding cells have the capacity for self-renewal, we subjected <i>Gli1<sup>CreER</sup>; Rosa26</i> mice to eight cycles of prostate involution and regeneration. Cells marked before castration expand after 8 cycles of involution/regeneration, indicating that the initially marked Shh-responding cells are self-renewing. Additionally, using <i>Gli1</i> null mutant mice, we demonstrate that <i>Gli1</i> is required to drive stromal expansion during prostate regeneration. Based on our results, we propose a model wherein Shh is expressed in adult prostate epithelial cells, the signal is received by the adjacent stroma, which responds by expressing critical genes, including the transcription factor <i>Gli1</i> , that result in expansion of the two stromal cell types.					
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## Introduction

The specific aims for the approved proposal are as follows:

**I. Determine the fate of Shh-responding cell populations in the mouse prostate**, using *Gli1-CreER* mice and GIFM.

*Hypothesis:* Shh directly regulates expansion of the glandular stroma but not the epithelial compartment during development, normal adult homeostasis and regeneration of the prostate.

**II. Ascertain whether *Gli1* is required to drive expansion of the stromal compartment of the prostate** using GIFM during development and androgen-mediated regeneration of the prostate in *Gli1* mutant mice.

*Hypothesis:* *Gli1* is required to drive stromal cell expansion.

**III. Assess the fate of Shh-responding cells during progression of prostate cancer and the role of *Gli1* in prostate cancer**, using GIFM and *Gli1* mutant mice with the TRAMP and other prostate cancer models.

*Hypothesis:* The epithelium becomes Shh-responsive during malignant transformation in prostate cancer and Shh-responding cells are enriched in metastatic tumors. Furthermore, *Gli1* null mutant prostates will form fewer and/or less aggressive tumors.

**IV. Determine whether there are populations of normal adult stem cells and/or cancer stem cells in the mouse prostate that respond to Shh**, using clonal analysis and Florescence Automated Cell Sorting (FACS).

*Hypothesis:* Stromal stem cells in the mouse prostate respond to Shh signaling, and epithelial cells that aberrantly respond to Shh signaling represent cancer stem cells.

The experiments pertaining to specific aims I and II are nearly complete. We are in the process of compiling data from these experiments to prepare a manuscript to submit for publication. Additionally, the mice required to conduct the experiments for specific aims III and IV have been generated, and we are waiting for these mice to mature to the appropriate ages to conduct the proposed experiments for these specific aims.

## Body

- Task 1. To determine the fate of Shh-responding cell populations in the mouse prostate (months 1-12).
- a. Genetic Inducible Fate Mapping (GIFM) studies during embryonic and early postnatal development, during normal adult homeostasis and during androgen mediated regeneration in the adult (n=6 animals for each experiment)
    - a. Tamoxifen gavage for development and homeostasis experiments
    - b. Castration, androgen pellet implantation and tamoxifen gavage for regeneration experiments.
  - b. Sectioning prostates from GIFM experiments and marker staining to determine which cell types are marked.
  - c. BrdU injections, TUNEL and Caspase staining to determine which cell types are proliferating and which cell types are dying during regeneration.

### Progress:

GIFM studies of Gli1-expressing cells during early postnatal development and during androgen-mediated regeneration in the adult are complete. Regarding the homeostasis experiments, we have analyzed several animals at six weeks and one year after tamoxifen gavage. We have found that there is a minimal, but statistically significant expansion at one year, as compared with six weeks.

Using cell type specific markers, we have determined conclusively that marked cells after development and regeneration are smooth muscle cells and fibroblasts and not epithelial cells. Continuing studies are ongoing to immunophenotype the initially marked cells

BrdU and Caspase staining indicates that there are dividing cells in the stromal and in the epithelial compartments during development and regeneration. We have shown that the marked cells (cells that are derived from the Gli1-lineage) are dividing proportionally less than non-marked stromal cells. Caspase staining indicates that there is cell death in both compartments during involution, following castration, and we have determined that marked cells undergo cell death, relative to non-marked stromal cells.

Task 2. Ascertain whether *Gli1* is required to drive expansion of the stromal compartment of the prostate (months 10-18).

- a. GIFM studies, using *Gli1* mutant mice during embryonic and early postnatal development, during normal adult homeostasis and during androgen mediated regeneration in the adult. (n=6 animals for each experiment)
  - a. Tamoxifen gauvage for development and homeostasis experiments
  - b. Castration, androgen pellet implantation and tamoxifen gauvage for regeneration experiments.
- b. Sectioning prostates from GIFM experiments and marker staining to determine which cell types are marked.
- c. BrdU injections, TUNEL and Caspase staining to determine which cell types are proliferating and which cell types are dying during regeneration.

Progress:

GIFM studies on late embryonic development and adult regeneration, using *Gli1* mutant mice are nearly complete. We changed the marking strategy in the same manner as described above for task 1. When we mark Gli1-expressing cells during postnatal development and follow their fate throughout development, we see less expansion than in wild-type mice. We are presently confirming these findings with additional mice. Furthermore, after regeneration, we see less overall stroma than in wild-type regenerated prostates as well as fewer marked cells. Preliminary BrdU and Caspase staining reveals less proliferation and more cell death in the stromal compartment in *Gli1* mutant mice, as compared to the stromal compartment of wild-type mice. We are in the process of confirming these results with additional mice.

Task 3. Assess the fate of Shh-responding cells during progression of prostate cancer and the role of *Gli1* in prostate cancer (months 1-36).

- a. Breed *Gli1*<sup>CreER</sup> mice on to a C57 background (months 1-12).
- b. Breed *Gli1*<sup>CreER</sup> ; *R26R* mice with *TRAMP* mice (months 1-18)
- c. Breed *Gli1*<sup>nLacZ</sup> mice on to a C57 background and then to the *TRAMP* mice (months 1-18)
- d. GIFM studies with *Gli1*<sup>CreER</sup> ; *R26R*; *TRAMP* mice (months 12-36)
  - a. Tamoxifen gauvage before onset of tumorigenesis.

- b. Tamoxifen gavage after tumorigenesis, before metastasis.
- c. Marker analysis of tumor tissue from a. and b. to determine whether marked cells contribute to primary tumors and metastatic tumors.
- e. Breed *Gli1* null mutants with *TRAMP* mice (months 1-12).
  - a. Analysis of tumor number and metastasis

Progress:

Analysis of TRAMP mice crossed to outbred mice indicates that these mice develop tumors with the same frequency as TRAMP mice on a C57/Bl6 inbred background. Therefore, we have decided to conduct our analysis on mixed C57/Bl6 and Swiss Webster outbred mice. In-situ hybridization experiments on tissue sections of metastatic tumors from various organs, taken from TRAMP mice, indicate increased expression levels of Shh, and Ihh. Additionally, we observe expression of *Gli1* in epithelial cells in these tumors.

Task 4. Determine whether there are populations of normal adult stem cells and/or cancer stem cells in the mouse prostate that respond to Shh (months 12-36).

- a. Clonal analysis of Shh-responding cells in the regenerating prostate (months 12-36) (n=6 animals for each experiment)
  - a. Involution/regeneration experiments described in task 1, with low-dose of tamoxifen

Progress:

As an additional test to determine if the Gli-expressing cells marked before castration are self-renewing stem cells, we have gavaged several mice with tamoxifen and then subjected these mice to more than 12 rounds of involution and regeneration. We reasoned that if these marked cells are true self-renewing stem cells, then the expansion should be maintained after several cycles of involution and regeneration. Initial analysis of mice cycled eight times indicates that the expansion of marked stromal cells is maintained after multiple cycles. These mice have now all been analyzed and we found that marking is in fact maintained at a high level after 12 rounds of involution/regeneration.

## Key Research Accomplishments

- Gli1 marks a population of stromal stem cells in the adult mouse prostate
  - Gli1-expressing cells, marked before castration, expand massively after regeneration, in the stroma and not in the epithelium.
  - Gli-expressing stem cells self renew, as indicated by the maintenance of this expansion after multiple rounds of involution/regeneration
  - Gli1-expressing cells are normally quiescent (slow cycling), as evidenced by the lack of expansion seen in the normal homeostatic prostate, analyzed one year after tamoxifen gavage.
- Gli1 marks a population of stromal stem cells in the developing prostate
  - Gli1-expressing cells marked during embryonic and postnatal development expand during development, in the stroma
- Stromal stem cell expansion of Gli1-expressing cells is dependent on *Gli1*
  - GIFM of Gli1-expressing cells, in *Gli1* mutants, show little or no expansion during development and during regeneration in the adult.
  - Overall stromal compartment is reduced after regeneration in *Gli1* mutant mice.



## Reportable Outcomes

### Abstracts:

**Levine CM** and Joyner AL: Genetic inducible Fate Mapping Uncovers the Behavior of Shh-Responding Cells in the Prostate.

(Poster presented at Building a Better Mouse II conference, July 2007, Nashville, TN)

The mammalian prostate is derived from two cellular lineages: an endodermally derived glandular epithelium and a mesodermally derived stroma composed of fibroblasts and smooth muscle cells. The secreted factor Sonic Hedgehog (Shh) is first expressed by urogenital sinus epithelial cells at E16.5. In response to Shh, the adjacent urogenital mesenchyme expresses *Gli1*, a transcriptional target of Shh signaling. As an approach to study the behavior of Shh-responding cells in the prostate, we used Genetic Inducible Fate Mapping (GIFM) to follow the fate of Shh-responding cells both during postnatal development and during androgen-mediated regeneration of the gland in the adult, two processes that are driven by stem or progenitor cell expansion. As *Gli1* expression is a sensitive readout of Shh signaling, we used a *Gli1*<sup>CreER</sup> allele and *Rosa26* reporter (Ahn & Joyner, Cell, 2004) to fate map Shh-responding cells. We show that the few stromal cells that are marked initially by GIFM at P14 or in the adult expand greatly during subsequent development (P14-P28) or during androgen-mediated regeneration, respectively. In both cases, the expansion of cells is confined to stromal fibroblasts and smooth muscle cells; no glandular epithelial cells are marked. These results indicate that *Gli1* either specifically marks stromal stem cells that expand during development and regeneration to give rise to the two stromal cell types, or that fibroblasts and smooth muscle cells in general have a high capacity for proliferation even in the adult prostate. Furthermore, using *Gli1* null mutant mice, we demonstrate that *Gli1* is required to drive stromal expansion during prostate regeneration. Based on our results, we propose a model wherein Shh is expressed in adult prostate epithelial cells, the signal is received by the adjacent stroma, which responds by expressing critical genes, including the transcription factor *Gli1*, that result in expansion of the two stromal cell types.

### Presentations:

New York University School of Medicine, 2008 MSTP retreat: Stem Cells in the Mouse Prostate Stroma Respond to Shh.

## **Conclusion**

We have completed the first two specific aims of the proposal. We have demonstrated that stromal stem cells in the normal adult prostate and in the developing prostate respond to Shh-signalling. We have evidence that these Shh-responding stem cells are normally quiescent, expand rapidly and massively during regeneration and development and that these cells are self-renewing. We have also demonstrated that the expansion of these Shh-responding stem cells is dependent on the transcription factor Gli1.

Additionally, we have begun our analysis of Shh-responding cells in tumor initiation, progression and metastasis. Preliminary analysis of metastatic tumors from TRAMP mice indicates that Shh, Ihh and Gli1 are all expressed at high levels in the epithelial cells of prostate cancer metastases. So far, we have not yet determined whether the same Shh-responding stromal stem cells in the normal adult prostate that undergo transformation and contribute to the epithelial tumors that develops in TRAMP mice. These studies are ongoing.